Conclusion: PGRN plays a chondroprotective role in the pathogenesis of OA, through a) inhibiting TNFα-induced catabolic and b) activating anabolic metabolism as a novel chondrogenic growth factor.

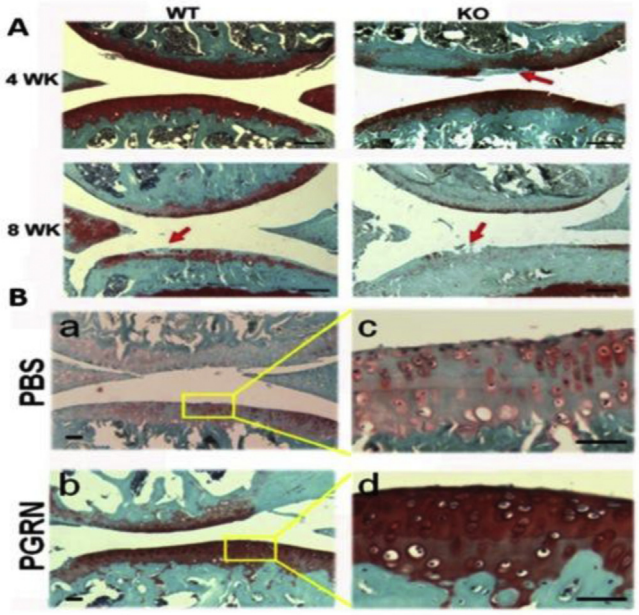

Fig. 3. The protective role of PRGN in surgically-induced osteoarthritis A) PGRN-/-mice showed elevated degradation of cartilage in DMM model. B) Intra-articular injection of PRGN reduces the loss of proteoglycan in surgically induced OA.

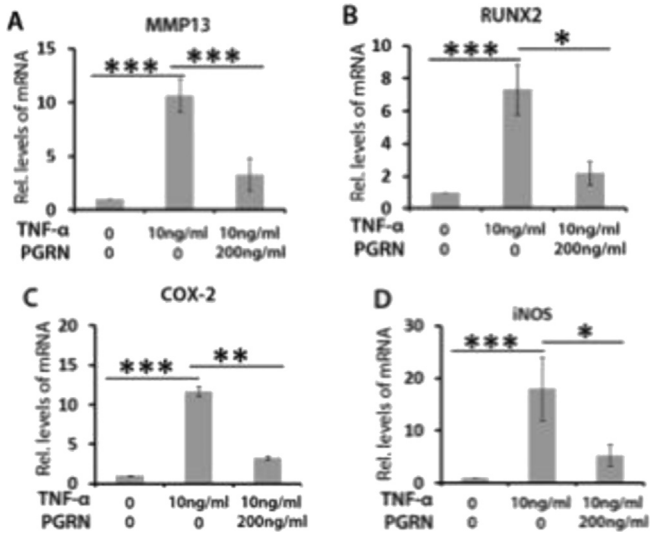

Figure 1. **PGRN effectively prevents the loss of cartilage by TNF-α.** Total RNA was extracted and real-time polymerase chain reaction (PCR) was performed using a sequence-specific probe and primers for MMP13 (a), RUNX2 (b), COX-2 (c) and iNOS (d). Each was tested under three conditions (control, TNF-α and TNF-α with PGRN). Our results demonstrated a drastic decrease in all metabolic levels with the induction of PGRN (Figure 4.a, b, c, d).

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TNFα IS A CRITICAL MEDIATOR OF SYNOVIAL HYPERPLASIA AND OSTEOPHYTE FORMATION IN HIGH FAT-FED MICE

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Purpose: Obesity and associated type 2 diabetes are important risk factors for osteoarthritis (OA). A critical hallmark of the metabolic disease of diabetes is a low-grade chronic inflammation. Evidence indicates that the proinflammatory cytokine TNFα plays an important role in mediating the metabolic dysfunction in this state. Inflammation is now also recognized to be a contributing factor in the pathogenesis of osteoarthritis. Here we use human fibroblast-like synoviocytes (FLS) and mouse models to test the hypothesis that TNFα plays an important role in mediating the adverse effects of obesity and type 2 diabetes on two aspects of OA progression: synovial hyperplasia and osteophyte formation.
Methods: Animals: Male C57BL/6J (wild type) mice were bred from existing colonies and age matched to TNFα-/- mice (Jackson Labs). Mice were placed on high-fat (HF, 60% kcal, D12492) or low-fat (LF, 10% kcal, D12450B) diets at 5 weeks of age (Open Source Diets, Research Diets). Mice were continued on their diets for 24 weeks before sacrifice and harvest. Histology: Following sacrifice, knee joints were isolated and formalin fixed, decalcified, and embedded in paraffin. Sagittal sections were stained with Alcian blue-hematoxylin before assessment. Synovial samples: Human FLSs were isolated from the synovial tissues of OA patients undergoing total knee arthroplasty. FLSs were serum-starved overnight prior to 24h treatment with cytokine and insulin. Total RNA was extracted from FLSs and gene expression quantitated by qRT-PCR.
Results: Immunohistochemistry demonstrated that while TNFα and BMP2 were not detected in the synovium of LF fed mice, there was strong synovial expression of both genes in mice fed the HF diet. This increased expression was associated with synovial hyperplasia and increased formation of osteophytes. To investigate these potential mediators of HF diet-associated osteoarthritis more directly, the response of human FLSs to TNFα was examined. In response to TNFα treatment, expression of BMP2 and TNFα was increased 6 and 40-fold, respectively, as measured by qRT-PCR. The latter result suggests a feed forward amplification of TNFα. Importantly, insulin inhibited the effects of TNFα on BMP2 expression by approximately 50%. To assess the in vivo effect of TNFα, TNFα knockout mice were placed on the HF and LF diets for 24 weeks. As expected, absence of TNFα blunted the weight gain and the glucose intolerance that is observed in HF diet fed mice. In LF fed TNFα-/- mice, osteophyte numbers and synovial hyperplasia were modestly increased above baseline, suggesting a role for TNFα in joint maintenance under control conditions. Importantly, the increase in osteophyte number and synovial hyperplasia present in HF-fed wild type mice was completely absent in HF TNFα-/- mice, supporting a pathogenic role for TNFα in osteoarthritis during HF diet consumption, obesity, and type 2 diabetes/systemic inflammation.
Conclusions: TNFα plays an important role in the weight gain and metabolic dysregulation of HF diet-associated obesity and type 2 diabetes. TNFα also promotes expression of osteoarthritis-associated cytokines by FLSs, which can be reversed by insulin treatment, suggesting a protective role for insulin in synovial joints. Finally, the TNFα-/- mouse model confirms the critical role of TNFα in the pathogenesis of synovial hyperplasia and osteophyte formation in HF-fed, obese and diabetic mice.

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SHORT BUT NOT LONG TERM SCLEROSTIN INHIBITION WORSENS POST-TRAUMATIC OSTEOARTHRITIS IN MICE

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Purpose: Sclerostin (SOST) and dikkopf-1 (DKK1) are secreted proteins that inhibit Wnt signaling. Increased Wnt-β-catenin activity is implicated in cartilage degradation in osteoarthritis (OA), at the same time driving increased bone formation (subchondral thickening, osteophytes) that typifies OA. This suggests that increasing SOST/DKK1 may be protective while inhibiting these Wnt antagonists may worsen OA cartilage and bone pathology. A recent report found that over-expression of DKK1 in chondrocytes did indeed reduce cartilage and bone pathology in post-traumatic (pt)OA in mice, however in another study systemic SOST inhibition did not alter ptOA cartilage erosion in rats. Whether these different outcomes relate to the inhibitor targeted and their systemic versus cell-specific regulation, or the species and ptOA model used is unclear. The present study examined whether systemic inhibition of SOST changed ptOA pathology in mice induced by the same meniscal destabilization (DMM) model as in the DKK1 over-expression study.
Methods: Forty-eight 10-week male C57BL6 mice had unilateral DMM surgery and were randomly assigned to receive 20mg/kg of anti-SOST or control IgG antibody (provided by Eli-Lilly) subcutaneously twice weekly: from the day of surgery and sacrificed at 4 (group 1) or 8 (group

3) weeks post-surgery; or from 4 weeks post-surgery until sacrifice at 8 weeks (group 2) (n = 8/antibody/group). At sacrifice knees were fixed, bone changes analysed by μCT, joints then decalcified and OA pathology scored (average of 2 blinded observers) on serial sagittal sections. SOST and DKK1 were immunolocalized in 3 mice of each group/antibody.

Results: Treatments were well tolerated with no adverse reactions observed; all animals completed the study. The significant effects of anti-SOST compared with IgG in operated limbs from the 3 groups are summarized in Table 1. Increased tibial cortical bone thickness and subchondral trabecular bone volume were seen by μCT in all groups with anti-SOST compared with IgG ($p<0.05$), confirming the efficacy of the SOST inhibition. However, subchondral bone plate thickness was only increased by anti-SOST in groups 1 and 2 ($p<0.05$). Similarly, histological scoring of subchondral bone sclerosis was only significantly increased with short-term SOST inhibition (groups 1 and 2). Inhibition of SOST only later in ptOA development (group 2) led to increased osteophyte size ($p<0.05$). Importantly, this later anti-SOST treatment (group 2) also increased articular cartilage structural damage ($p<0.05$). Immunohistochemistry revealed that anti-SOST antibody increased the number of SOST positive cells in calcified cartilage (x24-86%) and subchondral bone (x33-124%) in all groups. While short term SOST inhibition (groups 1 and 2) also increased DKK1 positive cells throughout the cartilage (x47-180%) and subchondral bone (x250-260%), long-term SOST blockade (group 3) reduced DKK1 positive cells in all tissues (to 30-60%).

Conclusion: Blocking SOST by systemic administration of a monoclonal antibody had detrimental effects on the progression of DMM-induced ptOA in mice. Importantly the effect was dependent on timing, being more pronounced with short-term administration and particularly when given later in disease. It is unclear whether the increase in cartilage damage (group 2), was a direct effect of anti-SOST on chondrocytes, inhibition of synovial SOST, or indirectly through changing subchondral bone. That long-term SOST-blockade did not alter joint pathology to the same extent as short-term treatment may be related to different compensatory changes in other Wnt-regulatory proteins as seen with DKK1. The temporal difference in SOST blockade in joint versus distant skeleton may reflect when SOST expression in OA is most pronounced and/or when increases in subchondral bone plate are most rapid or important in disease progression. The latter may also explain differences in our findings compared with those reported in rat ptOA, where more pronounced and prolonged bone loss occurs. Improved understanding of the regulation of Wnt signaling in different skeletal and joint tissues with time, may lead to novel therapeutic approaches in OA.

Table 1
Parameters significantly increased in anti-SOST compared to IgG treated mice (+ = $p < 0.05$, - = no change).

	Micro CT			Histology score		
	Cortical Bone	Subchondral Trabecular Bone	Subchondral Bone Plate	Subchondral Bone Plate	Osteophyte Size	Articular cartilage Erosion
Group 11-4 weeks	+	+	+	+	-	-
Group 24-8 weeks	+	+	+	+	+	+
Group 31-8 weeks	+	+	-	-	-	-

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IDENTIFICATION OF GREMLIN1 AS A CATABOLIC FACTOR INDUCED BY MECHANICAL STRESS LOADING IN ARTICULAR CHONDROCYTES

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Purpose: Excessive mechanical stress loading on articular cartilage is one of the major factors of osteoarthritis (OA); however, the molecular mechanisms of cartilage degradation by such loading remain unclear. The present study therefore analyzed gene expression profiles of articular chondrocytes under mechanical stress loading and investigated functions of the candidate gene.

Methods: To load mechanical stress on cultured chondrocytes, we used a programmable cell stretcher system, STB-140 (STREX). Primary articular chondrocytes from 5-day-old mouse knee joints were seeded onto stretch chambers at a concentration of 1.0×106 cells/chamber. After 48 h of culture on the chambers, we initially applied a uni-axial cyclic cell stretch (0.5 Hz, 10% stretch) to the articular chondrocytes for 30 min, then cultured them for an additional 24 h. cDNA microarray (SurePrint G3, Agilent Expression Array) analyses were performed using samples before and after the mechanical loading. The gene expressions were confirmed by real-time RT-PCR and immunohistochemistry. The catabolic ability was assessed by measuring the concentration of aggrecan released from cultured 3-week-old mouse femoral heads using the dimethylmethylene blue dye-binding assay. We used mouse primary articular chondrocytes and mouse chondrogenic cell line ATDC5 for further in vitro functional analyses.

Results: Microarray analyses revealed that 2,076 genes were increased more than twice by the mechanical stress loading. Among them, we focused on Gremlin1, which is one of the most highly expressed genes. Gremlin1 is known to be a secreted protein that regulates limb development as a BMP antagonist during skeletal development, although its function in articular cartilage is unknown. Immunohistochemistry revealed that Gremlin1 protein was localized in cytoplasm of mouse normal knee articular chondrocytes, and the expression was enhanced in the cells of the experimental mouse OA model. In the organ culture of mouse femoral heads, recombinant human (rh) Gremlin1 treatment increased aggrecan release into the medium in a dose-dependent manner. In primary culture of mouse articular chondrocytes, rhGremlin1 treatment induced the expressions of catabolic factors including Mmp13 and Adamts5, and suppressed those of anabolic factors including Col2a1, aggrecan and Sox9. Lentiviral overexpression of Gremlin1 in ATDC5 cells also induced the catabolic factor expressions and suppressed the anabolic factor expressions. In organ culture of mouse femoral heads, rhBMP-7 treatment decreased the aggrecan release, and this anti-catabolic effect was abolished by the co-treatment of rhGremlin1. To further identify other signals related to the catabolic effect of Gremlin1 under the mechanical stress, we performed pathway analyses and gene ontology analyses using the microarray data and Genespring software, and found the NF-κB signal to be the most potent candidate pathway associated with both mechanical stress loading and Gremlin1. To know the involvement of the NF-κB signal with the catabolic effect by Gremlin1, we used conditional knockout mice of Rela, a representative transcription factor of the NF-κB signal. In the organ culture of femoral heads from Col2a1-Cre;Relafl/fl mice, rhGremlin1 did not induce the aggrecan release, while it normally induced the release in the control Relafl/fl femoral heads. We further confirmed that suppression of the NF-κB signal by an IKK inhibitor (BMS-345541) treatment also diminished the increase of aggrecan release. Induction of catabolic factors and suppression of anabolic fators by rhGremlin1 were also diminished by the IKK inhibitor treatment.

Conclusion: We have identified Gremlin1 as a catabolic factor induced by the mechanical stress loaded to articular chondrocytes, not only through the BMP signal but also through the NF-κB signal. Gremlin1 may provide a novel therapeutic target of OA.

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A ROLE FOR TGFβ-RII/MCP-5 AXIS DURING POST TRAUMATIC OSTEOARTHRITIS AND POTENTIAL ROLE OF PTHRP IN MEDIATING MCP-5 EFFECT

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Purpose: In previous studies we found that the down-regulation of the chemokine MCP5 by TGF-β Type II receptor (TβRII) signaling is required for joint development. MCP-5 role in arthritis is demonstrated by human and animal studies. MCP-5, its human homologous MCP-1 and their sole common receptor CCR2, are increased in the inflamed joints